A Network Pharmacology and molecular docking-based study exploring the pharmacokinetics, safety and mechanism of action of Polyscias fulva bioactive compounds against uterine fibroids

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Abstract

Uterine Fibroids (UF) also known as uterine leiomyomas are a significant reproductive health challenge among the female population, globally. Apart from surgery which has several complications, many available pharmacological therapeutic options reduce symptoms rather than being curative. The use of *Polyscias fulva* for the management of UF by Traditionally in Uganda implored the scientific validation process through network pharmacology and molecular docking approaches. Using scholarly literature search, known bioactive compounds of *Polyscias fulva* were retrieved from various databases. The SwissADME platform was used to evaluate drug likeliness and pharmacokinetic parameters of the compounds. The potential target genes of the compounds were predicted using the Swiss Target Prediction Database. Human genes associated with UF were obtained from GeneCards and OMIM databases. The interaction between the compounds and UF genes was established through protein–protein interaction, gene ontology, and KEGG pathway enrichment analysis. The binding affinities between the bioactive compounds of *Polyscias fulva* and the retrieved UF hub targets were determined using AutoDock tools. Here we show that Five *Polyscias fulva* bioactive compounds: pinoresinol, lichexanthone, methyl atarate, β-sitosterol and Cauloside A exhibited drug likeness properties with moderate safety profiles. β -sitosterol demonstrated stronger binding affinity with five human uterine fibroids targets i.e. HIF1A (-9.21 kcal/mol), ESR1 (-8.31kcal/mol), EGFR (-9.75kcal/mol), CASP3 (-7.13kcal/mol) and CCND1(-5.74kcal/mol) while the other four compounds strongly bound to three targets (HIF1A, ESR1, EGFR). In conclusion, *Polyscias fulva* contains bioactive compounds with potential anti-proliferative activity against UF with promising pharmacokinetic properties and safety profiles using computational predictive models.

Introduction

Uterine leiomyomas (fibroids) are the most common pelvic tumours among women of reproductive age, affecting more than 70% of women worldwide (Yu et al., 2018). Several factors contribute to the growth and progression of fibroids, including; genetic, biological, and hormonal factors (Omar et al., 2019). Leiomyomas are often associated with several gynaecological problems such as dysmenorrhea, irregular pelvic pain and menorrhagia (Rice et al., 2012). The black women population has a higher prevalence of fibroids (18.5%) than other racial/ethnic groups (Egbe et al., 2018; Yu et al., 2018).

The estrogen signaling pathway is a key driver pathway in uterine fibroid development and comprises both genomic (direct and indirect effects of gene expression) and non-genomic factors, such as; the Ras-Raf-MEK (MAPK/ ERK kinase)-mitogen-activated protein kinase (MAPK), PI3K-phosphatidylinositol-3,4,5-trisphosphate (PIP3)-AktmTOR) pathways (the Ras-Raf-MEK-MAPK and PI3KPIP3-Akt-mTOR pathways, respectively (Borahay et al., 2017; Yang et al., 2022). Alterations in other critical pathways such as the WNT/β-catenin, TGF-β, growth factor–regulated signaling, ECM, YAP/TAZ, Rho/ROCK, and DNA damage repair pathways also play essential roles in uterine fibroids formation and development (Borahay et al., 2017). Notably, the development of uterine fibroids may be initiated and triggered by the interactions and crosstalk among these pathways.

Surgical procedures such as hysterectomy, myomectomy, uterine artery embolization (UAE) and magnetic resonance imaging-guided focused ultrasound surgery (MRgFUS) are recognized as the most definitive therapeutic interventions. However, the possibility of recurrence due to underlying risk factors exists (Donnez & Dolmans, 2016; Xu et al., 2021). Pharmacologically, non-steroidal anti-inflammatory drugs (aspirin, ibuprofen, and naproxen)(Giuliani et al., 2020), gonadotropin-releasing hormone analogs (leuprolide acetate, cetrorelix), synthetic antiprogestin steroids (mifepristone and asoprisnil), aromatase inhibitors, and selective progesterone receptor modulators (SPRMs) are currently used in management / treatment of UL (Lewis et al., 2018; Giuliani et al., 2020, Moroni et al., 2014). However, these drugs are associated with several adverse effects, short alleviation of symptoms, non-curative, and often many patients ultimately go for surgical alternatives, which are also associated with operative mortality and morbidity (Hodgson et al., 2017; Marjoribanks et al., 2016).

Polyscias fulva is one of the medicinal plants traditionally used in East and Central Africa in the management of uterine fibroids, cancer and other related conditions (Mbaveng et al., 2017). The therapeutic effects of *P. fulva* are thought to arise from various biologically active compounds. phytochemical studies performed on *P. fulva* stem bark yielded Eleven (11) compounds (Kuete et al., 2014). Six compounds were isolated from the ethyl acetate fraction and five from the n-butanol fraction. The compounds belonged to various chemical groups, but commonly saponins and triterpenoids (Njateng et al., 2015). Unlike conventional medicines, herbal medicines are multi-target and multi-component recipes whose bioactive components interact and modulate complex molecular and biological networks within the body to produce their specific therapeutic or toxic effects (Chandran et al., 2017; Pelkonen et al., 2014). Thus understanding the complex nature of how herbal medicines work is a challenge of every drug development programme. Novel and robust approaches to systematically investigate the pharmacokinetics, pharmacodynamics, and safety of herbal remedies are required (Yang et al., 2014).

Recent approaches emphasize the use of computer aided drug discovery (CAAD) to shorten the drug discovery process, and reduce cost of production (Chandershekar et al., 2020) as opposed to the traditional drug design and development approaches, which are complex, time consuming, and costly (Katiyar et al., 2012). Network pharmacology in conjunction with other several bioinformatic databases aid the expeditious establishment of relationships between drug-target-disease networks and associated pathways (Alves et al., 2022). The above advances coupled with molecular docking approaches that provide insight into the molecular interactions between ligands and targets at specific binding sites have enabled scientists to gain a detailed understanding of the specific interactions between bio-active compounds and genes associated with various diseases, thus accelerating the development of potential therapeutic interventions (Jakhar et al., 2020). In this study, we used the network pharmacology approach to explore the pharmacokinetics, safety, and mechanism of *Polyscias fulva* the bioactive compounds in the management of uterine fibroids.

Materials and methods

Bioactive compounds identification

A Scholarly Literature search for the identification of the bioactive compounds *Polyscias fulva* was conducted from literature repositories such as PubMed (https://pubmed.ncbi.nlm.nih.gov,accessed on 28th /October/2023), Springer (https://link.springer.com, accessed on 28th /October/2023) and Science Direct

(https://www.sciencedirect.com, accessed on 28th /October/2023).

Drug-likeness prediction, pharmacokinetics and toxicity screening of Polyscias fulva bioactive compounds

The *Insilico* screening methods were applied to evaluate the drug likeliness (DL) features and the pharmacokinetic parameters i.e. absorption, distribution, metabolism and excretion (ADME) properties of the bioactive compounds of *Polyscias fulva.* The Canonical smiles of all the identified bioactive compounds were uploaded on the SwissADME platform (http://www.swissadme.ch/ accessed on 29th /October/2023) and the default parameters were applied to perform the virtual screening. The different properties were calculated using the algorithms inbuilt within the software. SwissADME uses Caco2-cell (heterogeneous human epithelial colorectal adenocarcinoma cell lines) and MDCK (Madin-Darby Canine Kidney) cell models to predict oral drug absorption, skin permeability, human intestinal absorption, and transdermal drug absorption. Similarly, the program uses blood-brain barrier (BBB) penetration and plasma protein binding models to predict the distribution of the compounds. The Lipinski rule of five was applied and only those with not more than 1 violation were selected for further studies (Chen et al., 2020).

Prediction of Target Genes for the P. fulva Bioactive Compounds

The potential target genes of the *Polyscias fulva* bioactive compounds were predicted using the Swiss Target Prediction Database (http://www.swisstargetprediction.ch/) accessed on 5th /November/2023. The analysis of the target genes was limited to the *Homo sapiens* to ensure the relevance to human biology(Zhang et al., 2019). The final list was compiled after eliminating the duplicates.

Prediction of Target Genes in Uterine fibroids

The potential human target genes associated with uterine fibroids were harvested from the GeneCards (http://www.genecards.org/, accessed on 8th November 2023) and the Online Mendelian Inheritance in Man (OMIM; https://www.omim.org/, accessed on 8th November,2023) databases (Shamsol et al., 2023). The Key words “Uterine Fibroids”, “Uterine Leimyomas”, “Fibroids”, “Leimyomas” were used to conduct the search and any redundant genes were omitted ensure a comprehensive and non-duplicative list.

Construction of Compound-Disease-Target (C-D) Network

The target genes for the *P. fulva* bioactive compounds associated with uterine fibroids were obtained by analyzing both the obtained target genes of the compounds and the disease using the InteractiVenn (http://www.interactivenn.net/, accessed on 9th November, 2023). The overlapping genes were obtained and presented in form of a Venn diagram and a Compound-Disease network was constructed by integrating the data into the Cytoscape software (v 3.9.1) (https://cytoscape.org/) (Kalungi et al., 2023). The common target genes of both bioactive compounds of *P. fulva* and uterine fibroids were taken as the network nodes and the interconnections between these nodes were represented through connecting lines.

Construction and Analysis of Protein–Protein Interaction (PPI) Network

The STRING database Version 11.5 (https://string-db.org/, accessed on 15th November, 2023) was utilized to obtain a Protein-Protein Interaction network between the Target genes for the bioactive compounds in *P. fulva and* uterine fibroids. We limited the analysis to the *Homo sapiens* species proteins and those with confidence score of atleast 0.900 were selected for network visualization. The obtained network was exported to the Cytoscape software for visualization and analysis. The CytoHubba plugin was used to determine the significance of genes in the network by analyzing three parameters namely maximal clique centrality, maximum neighborhood component, and degree to identify the top 10 hub genes based on the scores. The results of the three parameters were imported to InteractiVenn (http://www.interactivenn.net/, accessed on 18 November 2023) and an intersect generating to obtain the final set of hub genes predicted to be the potential hub targets in the network (Ran et al., 2013; Shamsol et al., 2023).

Gene Ontology and Pathway Enrichment Analysis

To obtain a deeper understanding of the biological mechanisms associated with the combination of the bioactive compounds of *P. fulva* and its potential effect in uterine fibroids, a gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway enrichment analysis was performed using the using the Database for Annotation, Visualization, and Integrated Discovery (DAVID), version 2021 (https://david.ncifcrf.gov/home.jsp, accessed on 20 November 2023). The detailed information regarding the biological processes (BP), cellular components(cc), molecular functions (MF) and signaling pathways were retrieved by the software. The outcomes of the analysis were graphically presented as bar charts, with the top 15 from the GO analysis and KEGG analysis being shown. The p-values were calculated, and statistically significant results were indicated by a p-value of less than 0.05 (Kalungi et al., 2023; Shamsol et al., 2023).

Molecular Docking

Molecular docking studies were conducted to analyze the interactions between the bioactive compounds of *P. fulva* and the identified gene hubs. The three-dimensional (3D) structures of the target gene hubs; HIF1A (PDB ID: 1H2K, 2.15Å), ESR1 (PDB ID: 1A52, 2.8Å), EGFR (PDB ID: 1MOX 2.5Å), SRC (PDB ID:1A07, 2.2Å), TNF (PDB ID: 1A8M, 2.3Å), CASP3 (PDB ID; 1CP3, 2.3Å)CCND1 (PDB ID: 2W99, 2.8Å) were obtained from the RCSB Protein Data Bank (https://www.rcsb.org/, accessed on 21st November, 2023) as protein data bank (PDB) formats. These were read by AutoDock tools software (Version 1.5.7) and prepared as protein targets / receptors by adding polar hydrogen, removing water molecules, removing residual chains and ligands, adding Kollman charges and assigning atoms as AD4- type. The files were saved as .pdbqt extension.

The 3D structure of the *P. fulva* bioactive compounds; Pinoresinol (PubChem CID:73399), lichexanthone (PubChem CID:5358904), methyl atarate (PubChem CID:78335), beta sitosterol (PubChem CID:222284) and Cauloside A (PubChem CID:441928) were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/, accessed on 21st November,2023). The compounds were prepared as ligands by importing then into AutoDock tools software. The root and rotatable bonds were detected automatically and the ligand was saved as .pdbqt format. The binding conformation between the ligand

and receptor was predicted by AutoDockTools Version 4.2 (http://autodock.scripps.edu/ accessed on 12th November) and the binding energy (≤ − 5 kcal/mol), which is an outcome of molecular docking, was used to assess the potential of the ligand–receptor binding. Finally, the visualization of molecular interactions between the proteins and ligands was performed using Discovery Studio Visualizer 2021 (Kaur et al., 2017; Tao et al., 2020).

Results

Identified bioactive compounds of P. fulva

From the literature search, we retrieved eleven reported bioactive compounds of *P. fulva* with scientifically validated pharmacological activities as shown in Table 1.

Table I: Bioactive phytochemical compounds in P. fulva Araliaceae (Hiern) Harms

S/N	Compound names	CANONICAL SMILES	References
1	3-O-[α-L-arabinopyranosyl]-hederagenin (cauloside A)	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)CO)OC6C(C(C(CO6)O)O)O)C)C)C2C1)C)C(= O)O)C	(Mitaine-Offer et al., 2004)
2	Beta-Sitosterol	CCC(CCC(C)C1CCC2C1(CCC3C2CC = C4C3(CCC(C4)O)C)C)C(C)C	(Njateng et al., 2013)
3	Kalopanaxsaponin B	CC1C(C(C(C(O1)OC2C(OC(C(C2O)O)OCC3C(C(C(C(O3)OC(= O)C45CCC(CC4C6 = CCC7C8(CCC(C(C8CCC7(C6(CC5)C)C)(C)CO)OC9C(C(C(CO9)O)O)O)OC1C(C(C(C(O1)C)O)O)O)C)(C)C)O)O)O)CO)O)O)O	(Kuete et al., 2014; Mitaine-Offer et al., 2004)
4	Lichexanthone	CC1 = CC(= CC2 = C1C(= O)C3 = C(C = C(C = C3O2)OC)O)OC	(Mitaine-Offer et al., 2004)
5	Methyl atarate(Methyl 2,4-dihydroxy-3,6-dimethylbenzoate)	CC1 = CC(= C(C(= C1C(= O)OC)O)C)O	(Mitaine-Offer et al., 2004; Njateng et al., 2015)
6	Oleanolic acid	CC1(CCC2(CCC3(C(= CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1)C)C(= O)O)C	(Njateng et al., 2015)
7	Pinoresinol	COC1 = C(C = CC(= C1)C2C3COC(C3CO2)C4 = CC(= C(C = C4)O)OC)O	(Njateng et al., 2013)
8	Polyscioside A	CC1(CCC2(CCC3(C(= CCC4C3(CCC5C4(CCC(C5(C)C)OC6C(C(C(C(O6)C(= O)O)OC7C(C(C(C(O7)CO)O)O)O)O)OC8C(C(C(C(O8)CO)O)O)O)C)C)C2C1)C)C(= O)O)C	(Mitaine-Offer et al., 2004)
9	Quercetin-3-O-D-glucopyranoside	C1 = CC(= C(C = C1C2 = C(C(= O)C3 = C(C = C(C = C3O2)O)O)OC4C(C(C(C(O4)CO)O)O)O)O)O	(Bedir et al., 2001)
10	Sequalene	CC(= CCCC(= CCCC(= CCCC = C(C)CCC = C(C)CCC = C(C)C)C)C)C	(Bedir et al., 2001)
11	α-hederin	CC1C(C(C(C(O1)OC2C(C(COC2OC3CCC4(C(C3(C)C)CO)CCC5(C4CC = C6C5(CCC7(C6CC(CC7)(C)C)C(= O)O)C)C)C)O)O)O)O)O	(Bedir et al., 2001)

Drug-likeness, pharmacokinetics and toxicity profile of the retrieved bioactive compounds of P. fulva

The SwissADME screening results(Table 2) showed three compounds (Lichexanthone, Methyl atarate, Pinoresinol) are in conformity with the Lipinski's rule of five with zero violations indicating the good drug likeliness of these phytocompounds; Four compounds had one violation i.e. Beta-Sitosterol, cauloside A, oleanolic Acid and sequalene indicating a moderate drug likeness property of these compounds. Alpha-hederin, kalopanaxsaponin B, polyscioside A, quercetin-3-O-D-glucopyranoside had three violations indicating poor drug likeness properties of these compounds and henceforth were eliminated for the subsequent studies.

Table II: Drug-likeness, pharmacokinetics and toxicity prediction of the P. fulva Bioactive compounds

	Alpha-hederin	Beta-Sitosterol	Cauloside A	Kalopanaxsaponin B	Lichexanthone	Methyl atarate	Oleanolic Acid	Pinoresinol	Po A
Drug Likeliness property									
Molecular weight (< 500 g/mol)	750.96	414.71	604.81	1221.38	286.28	196.2	456.7	358.39	95
Polar surface area (PSA) (≤ 140 Å)	195.6	20.23	136.68	412.82	68.9	66.76	57.53	77.38	31
Rotatable bonds (< 10)	6	6	4	14	2	2	1	4	10
Hydrogen bond donors (no > 5)	7	1	5	15	1	2	2	2	11
Hydrogen bond acceptors (no > 10)	12	1	8	26	5	4	3	6	19
clogP (< 5)	2.92	7.19	3.89	-1.71	2.81	1.77	6.06	2.26	1.
Molecular refractivity	195.45	133.23	164.23	291.03	79.96	51.7	136.65	94.9	23
Lipinski violations	3	1	1	3	0	0	1	0	3
Bioavailability score	0.11	0.55	0.56	0.17	0.55	0.55	0.85	0.55	0.
Pharmacokinetic and toxicity properties									
Toxicity:									
Carcinogenicity(Value)Probability	(-) 0.97	-(0.97)	– (0.97)	(-)0.97	(-)0.91	(-)0.71	(-)0.99	(-)0.89	(-
Hepatotoxicity(Value)Probability	(-)0.93	+(0.61)	(-)0.86	(-)0.88	(-)0.56	(+)0.567	(+)0.8125	(-)0.75	(-
Skin sensitization(Value)Probability	(-)0.89	+(0.64)	(-)0.89	(-)0.89	(-)0.96	(-)0.867	(+) 0.5630	(-)0.75	(-
Cardiovascular toxicity(hERG) (Value)Probability	+(0.68)	– (0.47)	+(0.72)	(+) 0.77	(-)0.69	(-)0.66	(-)0.5287	(+)0.76	
LD50(mg/kg)	1500	890	1500	4000	3200	1900	2000	1500	32
Toxicity class	4	4	4	5	5	4	4	4	5
Absorption:									
Water solubility(Log S)	-4.23	-4.71	-4.05	-4.23	-3.33	-2.62	-4.39	-3.3	-4
Caco-2 Permeability(Value)Probability	(-)0.88	(+) 0.54	(-) 0.82	(-) 0.88	(+) 0.8919	(+) 0.7	(+)0.56	(+)0.57	0.
Plasma protein binding (%)	0.578	1	0.69	(0.58)	1.015	0.692	0.77	0.66	0.
GI absorption	Low	Low	Low	Low	High	High	Low	High	Lo
Log Kp (cm/s)	-8.3	-2.2	-6.62	-15.13	-5.58	-5.84	-3.77	-6.87	-1
Distribution:									
BBB permeant	No	No	No	No	Yes	Yes	No	Yes	No
Pgp substrate	Yes	No	Yes	Yes	No	No	No	Yes	Ye
Metabolism:									
CYP1A2 inhibitor	No	No	No	No	Yes	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	Yes	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	Yes	No	No	Yes	No
CYP3A4 inhibitor	No	No	No	No	Yes	No	No	Yes	No

CYP, cytochrome-p450; GI, gastrointestinal; BBB, blood–brain barrier; Pgp, P-glycoprotein; LD50, Lethal dose at 50%; Kp, skin permeation coefficient

Biological targets of P. fulva bioactive compounds and compound–disease target network construction (C-D network)

A total of two hundred seventy-five genes (275) were identified from GeneCards and OMIM databases as the target genes for the *P. fulva* bioactive compounds. We retrieved a total of six hundred five (605) target genes associated with uterine fibroids from GeneCards and OMIM databases. The IntersectVenn analysis showed a total of thirteen (13) genes intersecting between uterine fibroids and *P. fulva* bioactive compounds(Figure II).

The Cytoscape software was used to construct a compound-target network (Figure III) representing the interaction between *P. fulva* bioactive compounds and their potential molecular targets in relation to the management of uterine fibroids

Protein–Protein Interaction (PPI) Network

To demonstrate the interaction between the *P. fulva* bioactive compounds and their potential targets in the management of uterine fibroids, a protein to Protein Interaction network (PPI) analysis was performed (Figure IV). The STRING database was used to perform the analysis which was visualized using the Cytoscape software. A total of 48 nodes and 415 edges were established after hiding disconnected nodes that represented proteins and protein-protein associations respectively. The degree values of the nodes in the network were examined by the Network Analyzer plugin in Cytoscape and represented by the color of the circle with varying correspondences with an average degree value of 17.3. Remarkably, these highly interconnected nodes might be key targets for *P. fulva's* medicinal actions. The top 10 hub genes in the network were then identified using the CytoHubba plugin, which incorporates the maximal clique centrality (MCC), maximum neighborhood component (MNC), and degree algorithms. These genes were thought to be possible targets for *Polyscias fulva* in the treatment of Uterine fibroids. By finding the intersection of these three algorithms the seven hub genes were found i.e. (HIF1A, ESR1, EGFR SRC, TNF, CASP3, CCND1) (*Figure V*).

Gene ontology and Pathway Enrichment analysis

The gene ontology analysis (Figure VI) revealed that the top biological processes associated with the bioactive compounds of *P. fulva targets* were related to positive regulation of cyclin-dependent protein serine/threonine kinase activity, positive regulation of nitric-oxide synthase activity and positive regulation of transcription, DNA-templated. The cellular components analyses showed that the targets were primarily involved in the membrane raft, macromolecular complex, neuronal cell body and cytoplasm. The molecular function analyses showed the phytocompounds were associated with enzyme binding, ATPase binding and nitric synthase regulator activity. The KEGG pathway analysis (Figure VII) results indicated that several targets of the bioactive compounds of *P. fulva* against uterine fibroids were enriched in signaling pathways such as estrogen signaling pathways, proteoglycans in cancer, breast cancer and oxytocin signaling pathways. The analysis further showed that the bioactive compounds from *P. fulva* might play an anti-fibroid role through the estrogen signaling pathway. The potential targets and mechanism of action of the compounds are shown in figure VIII.

Molecular docking

The interactions between the selected five bioactive compounds of *P. fulva* (Pinoresinol, Lichexanthone, methyl atarate, Beta sitosterol and Cauloside) and the seven potential target genes (HIF1A, ESR1, EGFR SRC, TNF, CASP3, CCND1) were analyzed at a molecular level through molecular docking studies. We used the AutoDock software to perform the molecular docking studies. We obtained a total of 32 docking results and 15 results had binding energies below –5.0kcal/mol (Table III). All the five bioactive compounds of *P. fulva* had a binding affinity above 5.0kcal/mol with three target proteins i.e. HIF1A, ESR1 and EFGR. Beta sitosterol had a strong binding affinity with five target proteins with docking scores HIF1A; -9.21, ESR1; -8.31, EFGR; -9.75, CASP3; -7.13, CCND1; -5.74 indicating it plays a vital role in the treatment of uterine fibroids. The visualization of ligand target interactions with the lowest bind energies were performed and generated by Biovia Discovery Studio (Figure IX).

Table III: Binding energy between active compounds and seven core targets of P. fulva

Compound	Binding Energy(kcal/mol)						
	HIF1A	ESR1	EGFR	SRC	TNF	CASP3	CCND1
Pinoresinol	-7.1	-6.07	-5.73	-3.2	-3.3	-3.76	-4.52
Lichexanthone	-6.13	-5.86	-5.15	-3.91	-4.29	-4	-5
Methyl atarate	-5.27	-4.79	-3.92	-2.99	-3.69	-3.48	-3.58
Beta sitosterol	-9.21	-8.31	-9.75	-4.77	-4.88	-7.13	-5.74
Cauloside	-7.51	-6.52	-7.90	-2.9	-3.42	-4.3	-3.53

Discussion

The current therapeutic options for the management of uterine fibroids are associated with a myriad of shortcomings hence calling for a search for alternative options that are not only affordable and effective but also safe for the patient. In this study we investigated the safety, pharmacokinetics and mechanism of action of *P. fulva* bioactive compounds in the management of uterine fibroids using computer aided drug discovery approaches. The *in-vivo* activity of phytocompounds from herbal medicines is often compromised by their poor pharmacokinetic profiles ultimately affecting the therapeutic outcome (Kumar & Sharma, 2018). Gaining detailed insight into the pharmacokinetic characteristics of the bioactive compounds is critical in the evaluation of the therapeutic and toxicological effects of the herbal medicines(Yang et al., 2020).

Three compounds (methyl atarate [MW 286.26], lichexanthone [MW 196.2], pinoresinol [MW 258.39]) from *P. fulva* possessed acceptable physicochemical properties evidenced from the zero violations of Lipinski's Rule of five (RO5) and displaying appreciable bioavailability score (0.55) (Figure I). Implying these compounds could be well absorbed orally and thus act as potential oral anti-fibroid agents. Lichexanthone demonstrated a slightly higher Log Kp (cm/s) score (-5.58) indicating its higher ability to penetrate the cells compared to the two other compounds i.e. Methyl atarate (Log Kp (cm/s); -5.84) and pinoresinol (Log

Kp (cm/s; -6.87). The Log Kp parameter determines the ability of a compound to partition between different fluid phases i.e. the cells and the surrounding environment and is used as a predictor for the cell permeability of a substance (Avdeef, 2001; Ellison et al., 2020). The interaction between a compound with the cytochrome P450 enzyme system indicates how that compound can influence the metabolism of other drugs if co-administered (Delgoda & Westlake, 2004). The compound methyl atarate had no interactions with any of the CYP450 enzymes while the other compounds inhibited different metabolizing enzymes. This implies that with exception of methyl atarate, the other compounds can potentially interact with others co-administered with them which might result into increased plasma concentration (Daly et al., 2017) Thörn et al., 2011). This could cause toxicity incase the co-administered drug has a narrow therapeutic index. The toxicity prediction studies indicated that majority all the eleven bioactive compounds of *P. fulva* are relatively safe with LD50 above 1500mg/kg except alpha hederin that had an LD50 of 890mg/Kg. These findings imply that bioactive compounds of *Polyscias fulva* are relatively nontoxic when taken in a single high dose within 24 hours.

From the network pharmacology analysis, identified seven targets (HIF1A, ESR1, EFGR, SRC, TNF, CASP3 and CCND1) have been reported to play critical roles in the uterine fibroid pathophysiology. The Hypoxia-Inducible Factor (HIF1A) or dependent genes have been linked to uterine fibroids development (Fedotova et al., 2023; Ishikawa et al., 2019). Additionally HIF1A has been implicated in a number of human pathologies including gynecological diseases like preeclampsia, polycystic ovary syndrome, endometriosis and cancer and so many others (Fedotova et al., 2023). The estrogen receptor 1 (ESR1) gene that is mostly expressed in uterine tissues (Bakas et al., 2008; Borahay et al., 2017) is a key target for estrogen, a hormone involved in the pathogenesis of fibroids (Tang et al., 2019). The Epidermal growth factor receptor (EGFR) gene plays a major role in the process of cell growth and differentiation and also involved in proliferation and mutagenesis and the impairment in its function is associated with the development of many cancers and since about 28% of cases of endometrial cancer may be associated with myoma, the role of EGFR gene in the pathophysiology of Uterine fibroids, cannot be underscored (Ciarmela et al., 2011; Nikpey et al., 2018). Reports of augmented activity c-Src in the uterine leiomyoma model in wistar rats have been made(Borahay et al., 2015). The gene was associated with the formation of uterine leiomyomas in the animal model, whereas gestrinone markedly suppressed the growth of uterine leiomyomas in the model. The CCND1 is also a key regulatory protein in the cellular transition from the G1 to the S phase by triggering cyclin-dependent kinase (CDK) enzymes(Tchakarska & Sola, 2020). The increased expression of the gene disrupts the normal cell cycle control and affects the transition from the G1/S checkpoint of the cell cycle resulting into tumor development(Sun et al., 2008; Tchakarska & Sola, 2020). The over expression of the CCND1 protein is associated with cell proliferation and different types of cancer, including endometrial, cervical, lung and breast cancer(Elsheikh et al., 2008; Hosokawa & Arnold, 1998; Moreno-Bueno et al., 2003). A significant association between the CCND1 870AA genotype with UL susceptibility was observed in a population sample of women from the southeast of Iran(Salimi et al., 2017). Macrophages, present in UF, are responsible for producing TNF-α; a comparison between UF tumors to the nearby normal myometrium, showed an increased TNF-α expression in the former. TNF-α produced by adipocytes promotes the growth of UF and Thus far, it is known that polymorphisms in the genes that encode TNF-α, IL-6, and IL-1β have been linked to a higher risk these tumours(Ciebiera et al., 2018).

Molecular docking studies on the five bioactive compounds of *P. fulva* and the seven key targets of the hub genes indicated that all the five compounds bind strongly and stably to the active sites of three target genes i.e. HIF1A, ESR1 and EGFR with binding energies − 5.0 kcal/mol and hence could play a role in the management of uterine fibroids. Notably the compound beta sitosterol demonstrated the highest binding affinity on all the target proteins i.e. HIF1A (-9.21 kcal/mol), ESR1(-8.31kcal/mol), EGFR(-9.75kcal/mol), SRC(-4.77kcal/mol), TNF(-4.88kcal/mol), CASP3(-7.13kcal/mol) and CCND1(-5.74kcal/mol). β-sitosterol is a phytosterol whose inhibitory effect on the proliferation of human uterine leiomyoma cells has been investigated; The compound demonstrated a time dependant 16.7% inhibitory effect on the treated cells through arresting the subG1 phase related apoptosis (Park & Baek, 2005). The compound also increased the gene expression of p27 and p21 related cell cycle and decreased the expression of cyclin E-CDK2 complex. The time dependant decrease in the expression of pro-caspase 3 and PARP was also observed (Park & Baek, 2005). In another study; both β-sitosterol and stigmasterol demonstrated antitumor proliferation effect by reducing primary Human Uterine Leiomyoma (hUL) cell growth after 8-days treatment and suppress the growth of rat uterine leiomyoma ELT3 cells after 48hours of treatment (Lin et al., 2019). The phytosterols have demonstrated blood cholesterol-lowering activity, anticancer properties (with a beneficial effect on colon cancer growth inhibition), and anti-atherosclerotic, anti-inflammatory and antioxidative effects (Salehi et al., 2021; Trautwein & Demonty, 2007).

The KEGG pathway enrichment analysis revealed that uterine fibroids associated *Polyscias fulva* bioactive compounds targets are not only linked to the Estrogen signaling pathway but also associated with a number of other pathways that are closely associated with uterine fibroid development. Some of these include; Pathways in cancer, oxytocin signaling pathways, Breast cancer pathway, prolactin signaling pathways and endocrine resistance pathway. The estrogen signaling pathway is strongly associated with the pathobiology of uterine fibroids development(Borahay et al., 2015) and its reason as to why fibroids are regarded estrogen dependent due to clinical evidence of tumor regression during menopause or upon treatment with gonadotrophin-releasing hormone agonists(Lethaby & Vollenhoven, 2008). The pathway is sub classified into genomic pathway that depend on modulation of transcriptional activities through gene expression and the non-genomic pathways that are typically mediated through rapid activation of signaling cascades(Borahay et al., 2017; Luo et al., 2014). The direct genomic pathway involves the Estrogen- Estrogen receptors complex directly binding to the regulatory regions of the target genes to modulate gene expression whereas the indirect genomic pathway involves the estrogen-ER complex binding to DNA-binding TFs such as specificity protein 1, nuclear factor–κB, CCAAT/enhancer-binding protein β, GATA binding protein 1, and signal transducer and activator of transcription through protein-protein interaction resulting into the activation or repression of target gene expression in estrogen-sensitive tissue(Fuentes & Silveyra, 2019; Yaşar et al., 2017).

The non-genomic pathway involves the binding of the estrogen to its receptors such as mER, GPER1, and some subtypes of nuclear ERα and ERβ) to rapidly modulate signaling pathways(Borahay et al., 2015; Fuentes & Silveyra, 2019). Through the Ras–Raf–MEK–MAPK pathway and phosphatidylinositide 3-kinases (PI3K)–Akt through the PI3K–phosphatidylinositol-3,4,5-trisphosphate (PIP3)–Akt–mammalian target of rapamycin (mTOR) pathway, activation of downstream protein kinase pathways such the mitogen-activated protein kinase (MAPK) occurs which results into the indirect modulation of expression of certain genes(Castellano & Downward, 2011; Huang et al., 2021). There is overexpression of the Ras-Raf-MEK-MAPK pathway molecules in fibroids as compared to the myometrium(Borahay et al., 2015). Furthermore, the mRNA transcript levels of c-Fos and c-Jun (members of the downstream effectors of the

MAPK pathway) are lower in fibroids than in myometriums(Gustavsson, 2000; Lessl et al., 1997). Additionally, evidence implicating the aberrant PI3K–PIP3–Akt–mTOR pathway in fibroid pathogenesis is existent(Yang et al., 2022). For example, the upregulation of mTOR signaling in fibroids in humans and animal models has been described and reports of a higher expression of glycogen synthesis kinase-3 and cyclin D2 in fibroids than in myometriums(Borahay et al., 2015; Karra et al., 2010). More studies have further provided evidence of aberrant rapid estrogen signaling in leiomyoma. In both myometrial and fibroid cells, estrogen increases protein kinase Cα within minutes. However, phosphorylated MAPK is increased in fibroid cells but not in myometrial cells(Nierth-Simpson et al., 2009).

Our findings indicate that β-sitosterol is a promising bioactive compound of *P. fulva* in the management of uterine fibroids as evidenced from its high binding energy with all the target hub genes. However, the other five compounds could also be potential agents in the management of uterine fibroids by targeting the HIF1A, ESR1 and EGFR genes due to the high binding energy exhibited. The findings provide the initial evidence for the ant-fibroid activity of *P. fulva* and validate the traditional use of *P. fulva* in East Africa by Traditional medicinal practitioner for the management of uterine fibroids.

Conclusion

The findings of this study show that the five bioactive compounds from *Polyscias fulva* i.e. pinoresinol, lichexanthone, methyl atarate, β-sitosterol and Cauloside A have drug likeness properties with moderate safety profiles and could play an important role in the management of uterine fibroids through interaction with the key biological targets i.e. HIF1A, ESR1 and EFGR with β -sitosterol exhibiting a stronger binding affinity with other targets like CASP 3 and CCND1. However, it's worth noting that the investigations were conducted in *in-silico.* Hence more validation studies using *in vitro* and *in vivo* modes are recommended to further authenticate our findings.

Declarations

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Author contributions

KK conceptualized and conducted the study under the guidance and supervision of YG, LM and EG. KK wrote the first draft of the manuscript and reviews were made by YG, SBO, LM and EG. All authors have read and approved the manuscript.

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Data availability

Data are available upon request from the authors.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figures

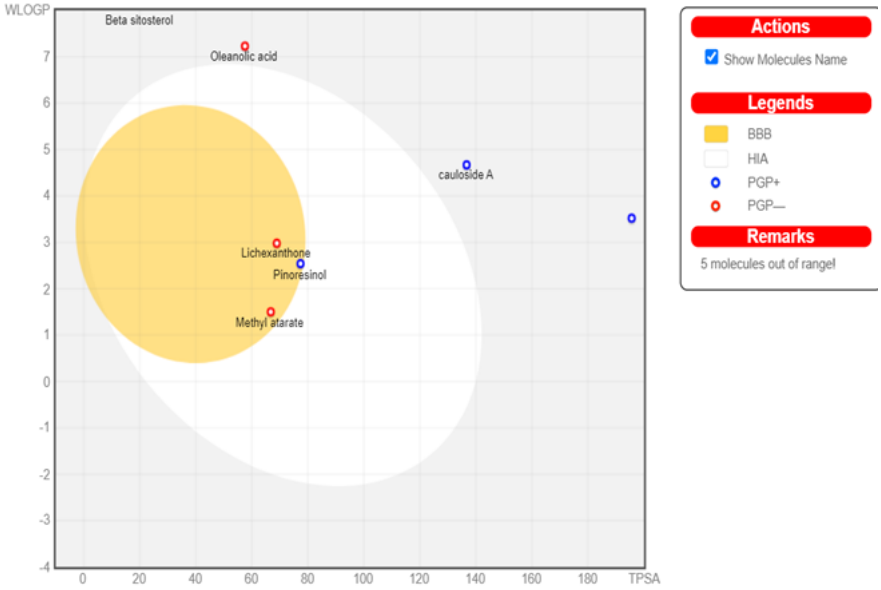

Figure 1

The BOILED EGG showing the Bioavailability of the different bioactive compounds from Polyscias fulva

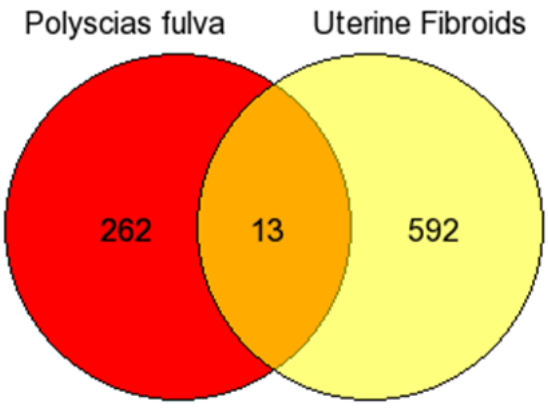

Figure 2

The potential targets of six bioactive compounds of P. fulva against uterine fibroids

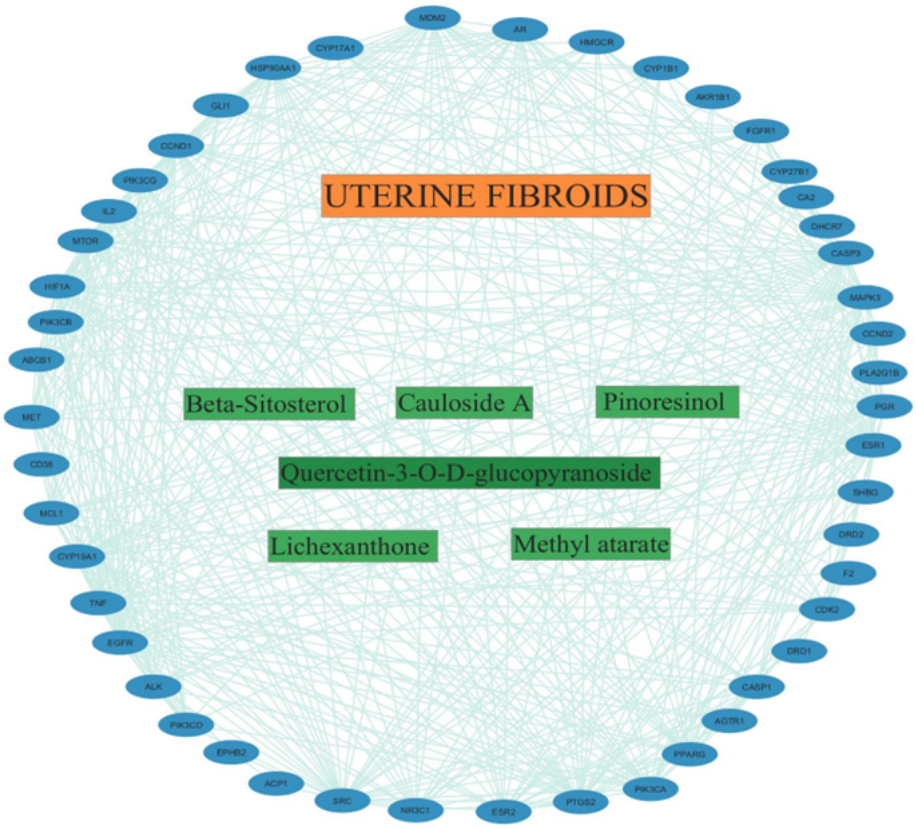

Figure 3

The compound-target network of P. fulva bioactive compounds against uterine fibroids using Cytoscape. The blue nodes represent the interacting target genes between the compound (green nodes) and target (orange node)

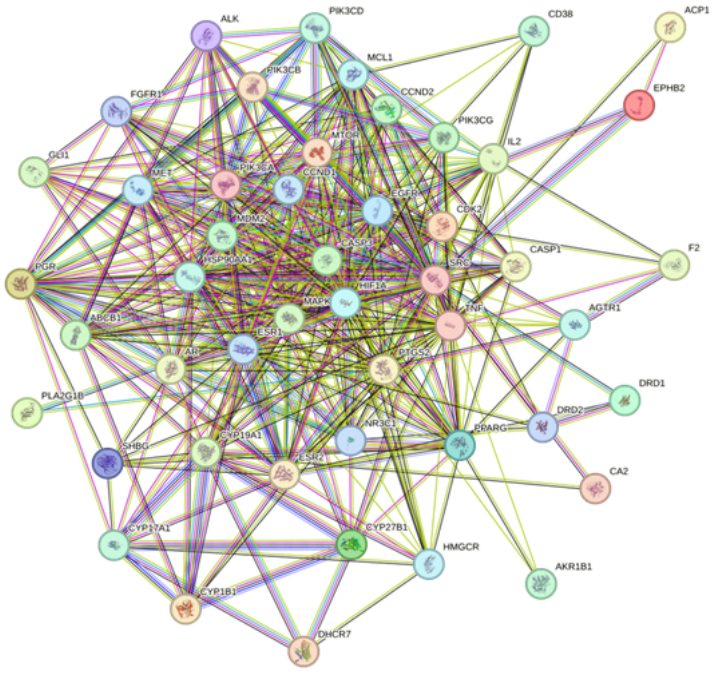

Figure 4

Protein to protein interactions of the Uterine Fibroids-Polyscias fulva bioactive compounds associated targets generated using STRING

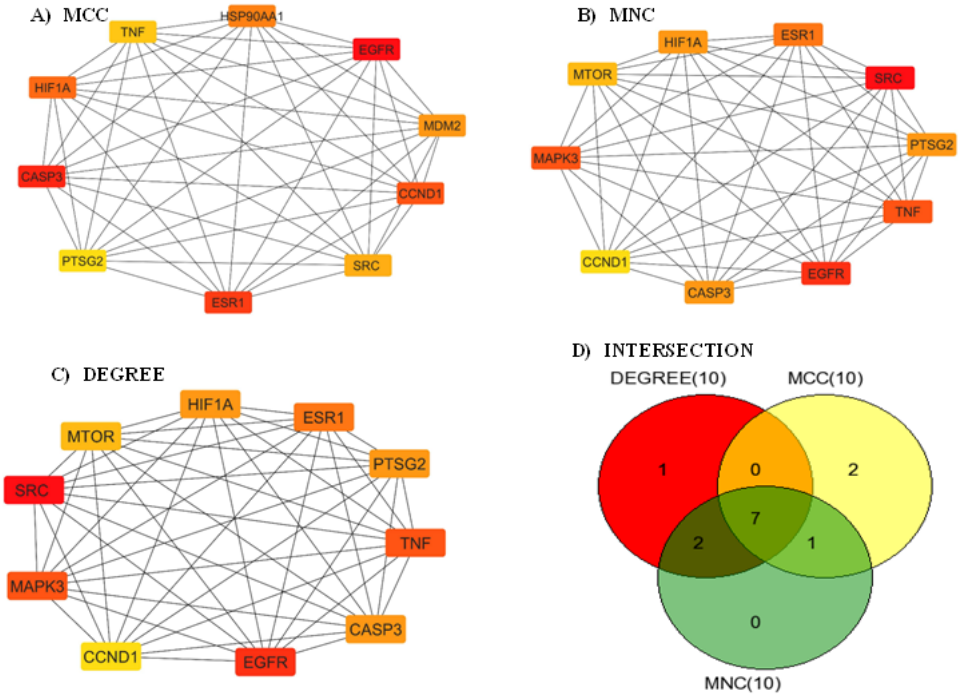

Figure 5

The top ten hub gene networks of P. fulva bioactive compounds against uterine fibroids by incorporating three algorithms—(A) maximal clique centrality (MCC), (B) maximum neighborhood component (MNC), (C) degree of nodes, and (D) The Venn diagram illustrates seven hub genes screened by the intersections of the three algorithms. The genes with the highest values are considered the most important hub genes and are depicted with a dark red color. Conversely, genes with lower values were considered less significant and are depicted with a light-yellow color, indicating the ranking position of each gene in the network.

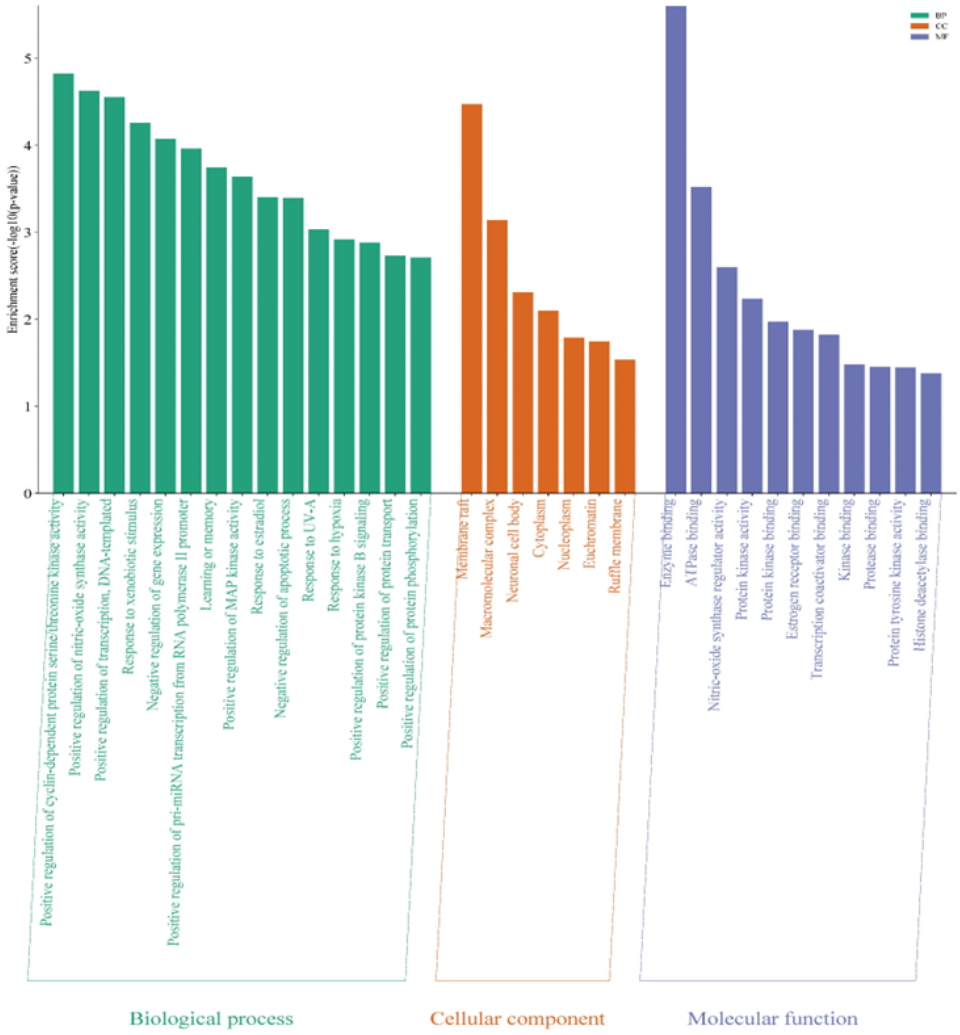

Figure 6

Gene ontology (GO) enrichment analysis. The bar chart represents the most significantly enriched GO terms (−log10 (p-value) in biological processes, cellular components, and molecular function, comprising the top 15 terms related to the target genes.

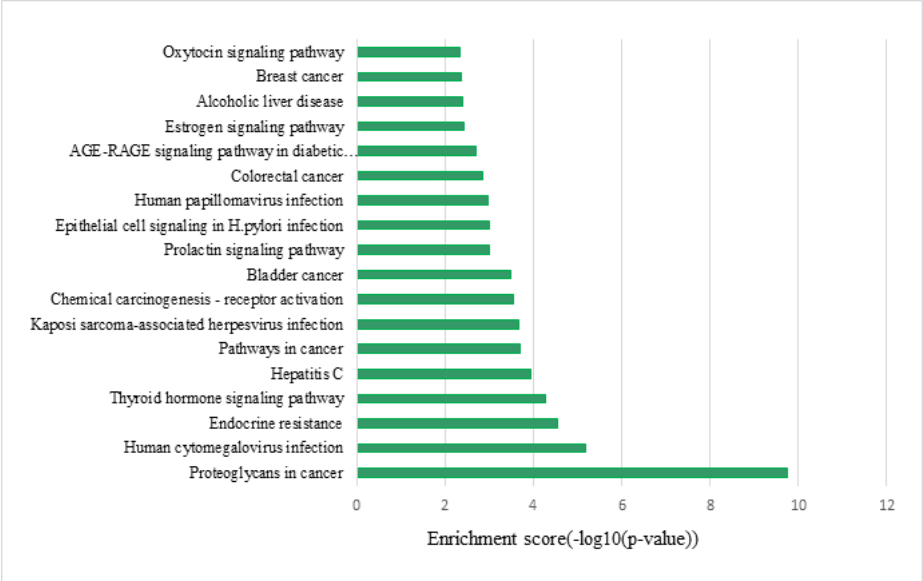

Figure 7

KEGG pathway enrichment analysis. The bar chart visualizes the top 15 enriched KEGG pathways of P. fulva bioactive compounds against uterine fibroids

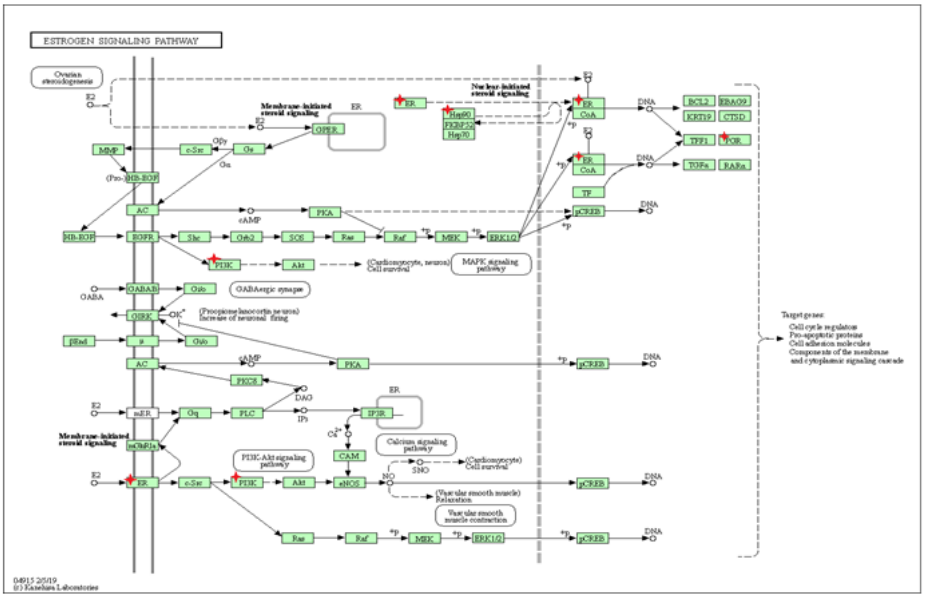

Figure 8

Potential targets and mechanism of bioactive compounds in P. fulva against Uterine fibroids. Red stars represent targeted genes involved in the estrogen signaling pathway

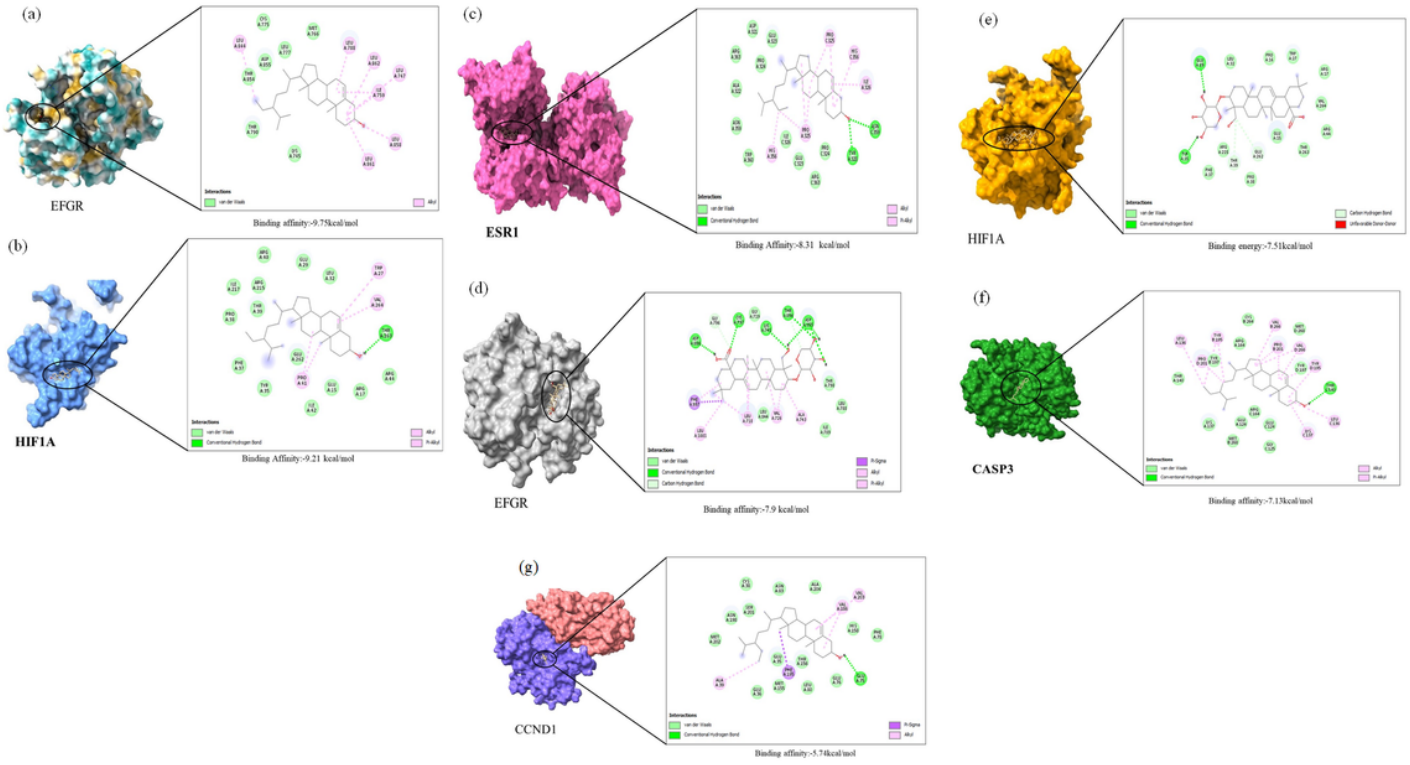

Figure 9

Molecular docking results of the lowest binding energy in each target with the bioactive compound of P. fulva. (a) EFGR-Beta sitosterol, (b) HIF1A-Beta sitosterol, (c) ESR1-Beta sitosterol, (d) EGFR-Cauloside, (e) HIF1A-Cauloside, (f) CASP3-Beta sitosterol and (g) CCND1-Beta sitosterol.